POLYPEPTIDES AND THEIR USES

FIELD OF THE INVENTION

The present invention relates to polypeptide compounds and to their use in medicine. Compounds according to the invention are believed to be potentially useful as cognitive enhancers and in the treatment of neurodegenerative diseases such as Alzheimer's disease.

BACKGROUND TO THE INVENTION

Alzheimer's disease is a degenerative brain disease which is characterised by progressive loss of memory and subsequently most other cognitive functions in an irreversible decline over a period of years. It represents a substantial health problem, particularly in an ageing population.

The amyloid precursor protein ("APP") is a multifunctional transmembrane protein and is known to have important functions in normal brain tissue. The human form of APP is known to consist of 695 amino acid residues (SEQ ID No: 1) in a sequence which is also known (see Kang et al, Nature 325, 733-736 (1987), the contents of which are incorporated herein by reference). The chick form of APP is known to consist of 534 amino acid residues (SEQ ID No: 2) and to resemble the human form closely, being approximately 95% homologous therewith (see the paper by Kang et al just mentioned and Barnes et al, J Neurosci, 18 (15) 5869-5880 (1998), contents of which are also incorporated herein by reference). The amino acid sequences of the human and chick forms of APP are reproduced in figure 1 of the drawings of this specification.

Two effects which have been noted to take place in the brain of a person suffering from Alzheimer's disease are the build up outside the nerve cells of the brain of

tangled masses of protein and the build up inside the brain cells of a different protein. The extracellular proteins are known to be aggregates of polypeptides having amino acid sequences corresponding to portions of the extracellular part of APP. The tangled masses of these proteins are known as amyloid plaques. The intracellular proteins are known as tau proteins. It is however not known whether either or both of the extracellular accumulation of amyloid plaques and the intracellular accumulation of tau proteins are the causes or the symptoms of Alzheimer's and related neurodegenerative diseases of the Alzheimer type.

The amino acid sequence of the β-amyloid polypeptide fragment (1-42) is identical in the human and chick forms of APP and consists of amino acid residues 597 to 638 in the human form and residues 436 to 477 in the chick form, (see the papers by Kang et al and Barnes et al referred to hereinbefore).

DEFINITIONS

The following expressions are used in this specification and have the following meanings:

APP	means "amyloid precursor protein";
human APP	means the human form of APP;
chick APP	means the chick form of APP;
RERMS	means the pentapeptide Arg-Glu-Arg-Met-Ser (SEQ ID No: 3);
APP 328-332	also means the pentapeptide Arg-Glu-Arg-Met-Ser (SEQ ID No: 3) which corresponds to amino acid residues 328 to 332 of human APP ;
SMRER	means the pentapeptide Ser-Met-Arg-Glu-Arg (SEQ ID No: 4);
Aβ domain	means the domain of APP which

forms β-amyloid plaques;

β-amyloid 12-28 means the sequence of amino acid residues which constitute part of the Aβ domain of human APP, the sequence being Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys(SEQ ID No: 8) which corresponds to amino acid residues 608 to 624 of human APP and amino acids 447 to 463 of chick APP;

RSAER means the pentapeptide Arg-Ser-Ala-Glu-Arg (SEQ ID No: 5); and

APP 319-335 means the polypeptide Ala-Lys-Glu-Arg-Leu-Glu-Ala-Lys-His-Arg-Glu-Arg-Met-Ser-Gln-Val-Met (AKERLEAKHRERMSQVM)(SEQ ID No: 6).

RER means the tripeptide Arg-Glu-Arg (SEQ ID No: 9).

RERM means the tetrapeptide Arg-Glu-Arg-Met (SEQ ID No: 10).

MRER means the tetrapeptide Met-Arg-Glu-Arg (SEQ ID No: 11).

Amino acid as used herein is meant to include both natural and synthetic amino acids, and both D and L amino acids.

Standard amino acid means any of the twenty standard L-amino acids commonly found in naturally occurring peptides.

Nonstandard amino acid means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or derived from a natural source. As used

herein, "synthetic amino acid" also encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the peptides of the present invention, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups which can change the peptide's circulating half life without adversely affecting their activity. Additionally, a disulfide linkage may be present or absent in the peptides of the invention.

Derivative includes any purposefully generated peptide which in its entirety, or in part, has a substantially similar amino acid sequence to the present compounds. Derivatives of the present compounds may be characterized by single or multiple amino acid substitutions, deletions, additions, or replacements. These derivatives may include (a) derivatives in which one or more amino acid residues of the present compounds are substituted with conservative or non-conservative amino acids; (b) derivatives in which one or more amino acids are added to the present compounds;

(c) derivatives in which one or more of the amino acids of the present compounds include a substituent group; (d) derivatives in which the present compounds or a portion thereof is fused to another peptide (e.g., serum albumin or protein transduction domain); (e) derivatives in which one or more nonstandard amino acid residues (*i.e.*, those other than the 20 standard L-amino acids found in naturally occurring proteins) are incorporated or substituted into the present compounds sequence; and (f) derivatives in which one or more nonamino acid linking groups are incorporated into or replace a portion of the present compounds.

Throughout this specification and its claims amino acid sequences are written using the standard one-letter or three-letter abbreviations. All sequences are written from left to right in the direction from the N-terminal to the C-terminal.

The following term is defined as follows:

reverse order sequence as used herein, the reverse order sequence of a given sequence is a sequence in which the order of amino acid residues is reversed compared with the given sequence when reading in the direction from the N-terminal to the C-terminal and *vice versa*. Thus, for example, SMRER is the reverse

order sequence of RERMS, each being read as stated above from left to right in the N-terminal to C-terminal direction. Further, MVQSMRERHKAELREKA (SEQ ID No: 7) is the reverse order sequence of APP 319-335 defined above.

SUMMARY OF THE INVENTION

The present invention provides a compound having a formula comprising:

$$X_1\text{-Arg-Xaa-Arg-}X_2 \qquad (I)$$

wherein X_1 and X_2, which may be the same or different, each represents from zero to 30 natural or synthetic amino acid residues or derivatives thereof and Xaa represents a natural or synthetic amino acid or a derivative thereof. Xaa is preferably glutamic acid.

The present invention also provides a compound having a formula comprising:

$$X_1\text{-Arg-Xaa}_1\text{-Arg-Xaa}_2\text{-Xaa}_3\text{-}X_2 \qquad (II)$$

wherein X_1 and X_2, which may be the same or different, each represents from zero to 30 natural or synthetic amino acid residues or derivatives thereof and Xaa_1, Xaa_2 and Xaa_3, which may be the same or different, each represents a natural or synthetic amino acid or a derivative thereof.

In both compound (I) and compound (II), amino acid derivatives include, for example, substituted amino acids.

In both compound (I) and compound (II), X_1 and X_2 are each preferably from zero to 20, more preferably from zero to 10.

When X_1 and X_2 are both zero in formula (I), formula (I) is that of a tripeptide which is Arg-Glu-Arg (RER) when

Xaa is glutamic acid. When X_1 and X_2 are both zero in formula (II), formula (II) is that of a pentapeptide which is RERMS when Xaa_1 is glutamic acid, Xaa_2 is methionine and Xaa_3 is serine.

In a compound according to formula (II) Xaa_1 is preferably glutamic acid, Xaa_2 is preferably methionine and Xaa_3 is preferably serine.

Preferably, compounds according to the invention are compounds in which X_1 and X_2 are such that (I) or (II) represents an amino acid sequence which is identical or closely homologous to amino acid residues 328 to 332 of human APP and up to 30 successive amino acid residues of human APP extending in each direction therefrom.

It is also preferred that compound (I) is one in which X_1 and X_2 are such that the formula represents a reverse-order amino acid sequence which is identical or closely homologous to amino acid residues 330 to 328 of human APP and up to 30 successive amino acid residues of human APP extending in each direction therefrom.

As used herein, a peptide or a portion of a peptide which is "closely homologous" means the peptide, or the portion thereof, has an amino acid homology of greater than about 80% with respect to a reference peptide, preferably greater than about 90% and, more preferably, greater than about 95%.

Amino acid sequence homology may be computed by using the BLASTP and TBLASTN programs which employ the BLAST (basic local alignment search tool) 2.0.14 algorithm; BLASTP and TBLASTN settings to be used in such computations are indicated in Table 1 below. Amino acid sequence identity (complete homology) is reported under "Identities" by the BLASTP and TBLASTN programs. Amino acid sequence similarity (degree of homology) is reported under "Positives" by the BLASTP and TBLASTN programs. Techniques for computing amino acid sequence homology are well known to those skilled in the art, and the use of the BLAST algorithm is described in Altschul et al. (1990), *J. Mol.*

Biol. 215: 403-10 and Altschul et al. (1997), *Nucleic Acids Res.* 25:3389-3402, the disclosures of which are herein incorporated by reference in their entirety. BLASTP and TBLASTN programs utilizing the BLAST 2.0.14 algorithm and may be accessed at http://www.ncbi.nlm.nih.gov/.

Table 1 - Settings to be used for the computation of amino acid sequence similarity or identity with BLASTP and TBLASTN programs utilizing the BLAST 2.0.14 algorithm

Expect Value	10
Filter	Low complexity filtering using SEG program*
Substitution Matrix	BLOSUM62
Gap existence cost	11
Per residue gap cost	1
Lambda ratio	0.85
Word size	3

*The SEG program is described by Wootton and Federhen (1993), *Comput. Chem.* 17: 149-163.

The invention also provides compounds having a formula comprising:

$$X_1\text{-}Xaa_3\text{-}Xaa_2\text{-}Arg\text{-}Xaa_1\text{-}Arg\text{-}X_2 \qquad \text{(III)}$$

wherein X_1, X_2, Xaa_1, Xaa_2 and Xaa_3 are as stated hereinbefore.

When X_1 and X_2 are both zero, the formula is that of a pentapeptide which is SMRER when Xaa_1 is glutamic acid, Xaa_2 is methionine and Xaa_3 is serine.

Such formulae represent the reverse-order sequences of the formulae mentioned hereinbefore.

Preferably, the compound is one in which X_1 and X_2 are such that the formula represents a reverse-order amino acid sequence which is identical or closely homologous to amino acid residues 332 to 328 of human APP and up to 30 successive amino acid residues of human APP extending in

each direction therefrom.

As used herein, a peptide or a portion of a peptide which is "closely homologous" means the peptide, or the portion thereof, has an amino acid homology of greater than about 80% with respect to a reference peptide, preferably greater than about 90% and, more preferably, greater than about 95%.

Preferably, X_1 in (I) represents:

X_3-Ala-Lys-Glu-Arg-Leu-Glu-Ala-Lys-His

and/or X_2 represents

Met-Ser-Gln-Val-Met-X_4

X_3 and X_4 being the same or different and representing from zero to 30 natural or synthetic amino acid residues or derivatives thereof.

X_3 and X_4 are again preferably each from zero to 20, more preferably from zero to 10.

When X_3 and X_4 are both zero and Xaa is glutamic acid, the formula corresponds to the sequence of amino acid residues 319 to 335 of human APP.

It is also preferred that X_1 in (I) represents:

X_3-Met-Val-Gln-Ser-Met

and/or X_2 represents:

His-Lys-Ala-Glu-Leu-Arg-Glu-Lys-Ala-X_4

wherein X_3 and X_4, which may be the same or different, each represents from zero to 30 natural or synthetic amino acid residues or derivatives thereof.

X_3 and X_4 are again preferably each from zero to 20, more preferably from zero to 10.

When X_3 and X_4 are both zero and Xaa is glutamic acid,

the formula corresponds to the reverse-order sequence of amino acid residues 335 to 319 of human APP.

Preferably, X_1 in (II) represents:

X_3-Ala-Lys-Glu-Arg-Leu-Glu-Ala-Lys-His

and/or X_2 represents

Gln-Val-Met-X_4

X_3 and X_4 being the same or different and representing from zero to 30 natural or synthetic amino acid residues or derivatives thereof.

X_3 and X_4 are again preferably each from zero to 20, more preferably from zero to 10.

When X_3 and X_4 are both zero and Xaa_1, Xaa_2 and Xaa_3 are glutamic acid, methionine and serine, respectively, the formula corresponds to the sequence of amino acid residues 319 to 335 of human APP.

Preferably, X_1 in (III) represents:

X_3-Met-Val-Gln

and/or X_2 represents:

His-Lys-Ala-Glu-Leu-Arg-Glu-Lys-Ala-X_4

wherein X_3 and X_4, which may be the same or different, each represents from zero to 30 natural or synthetic amino acid residues or derivatives thereof.

X_3 and X_4 are again preferably each from zero to 20, more preferably from zero to 10.

When X_3 and X_4 are both zero and Xaa_1, Xaa_2 and Xaa_3 are glutamic acid, methionine and serine, respectively, the formula corresponds to the reverse-order sequence of amino acid residues 335 to 319 of human APP.

The invention also provides compounds having the

formula (I) in which Xaa is glutamic acid and either X_1 is methionine and X_2 is zero, or X_1 is zero and X_2 is methionine. These are the compounds Met-Arg-Glu-Arg (MRER) (SEQ ID No: 11) and Arg-Glu-Arg-Met (RERM) (SEQ ID No: 10), respectively.

In addition to the compounds mentioned hereinbefore, the present invention also provides the compounds (including RER, RERMS and SMRER) for use in medicine and their use in the preparation of medicaments for the treatment of neurodegenerative diseases, including Alzheimer's disease, and as cognitive enhancers.

The invention further provides pharmaceutical compositions comprising the compounds (including RER, RERMS and SMRER) and a pharmaceutically-acceptable carrier and also compositions in which a compound according to the invention (including RER, RERMS and SMRER) is chemically or physically linked to a further molecule or vehicle to form a complex for pharmaceutical delivery of the compound.

The compounds which are most preferred in the medical uses and pharmaceutical compositions are the following:

Arg-Glu-Arg (SEQ ID No: 9)

which corresponds to amino acid residues 328-330 of human APP,

Arg-Glu-Arg-Met-Ser (SEQ ID No: 3)

which corresponds to amino acid residues 328-332 of human APP,

Ser-Met-Arg-Glu-Arg (SEQ ID No: 4)

which is the reverse-order polypeptide of the above,

Ala-Lys-Glu-Arg-Leu-Glu-Ala-Lys-His-Arg-Glu-Arg-Met-Ser-Gln-Val-Met (SEQ ID No: 6)

which corresponds to amino acid residues 319-335 of human

APP, and

Met-Val-Gln-Ser-Met-Arg-Glu-Arg-His-Lys-Ala-Glu-Leu-Arg-Glu-Lys-Ala (SEQ ID No: 7)

which is the reverse-order polypeptide of the above.

The invention further provides a compound having a formula comprising

X_1-Ser-Met-Arg-Glu-Arg-X_2 (IV)

wherein X_1 and X_2, which may be the same or different, each represents from zero to 30 amino acid residues, the amino acid residues of X_1 and X_2 being such that, when X_1 and X_2 are not both zero, the formula represents a reverse-order sequence corresponding to amino acid residues 332 to 328 of human APP and from zero to 30 successive amino acid residues of human APP extending in each direction therefrom, the formula also comprising sequences closely homologous to said reverse-order sequence and sequences in which said amino acids thereof are replaced by nonstandard amino acids and/or by derivatives of said amino acids, provided always that the compound is not

Met-Val-Gln-Ser-Met-Arg-Glu-Arg-His-Lys-Ala-Glu-Leu-Arg-Glu-Lys-Ala (SEQ ID No: 7)

Subject to the above proviso, formula (IV) thus includes within its scope polypeptides which consist of a core sequence of the five amino acid residues 332 to 328 of human APP in reverse order relative to human APP and, extending therefrom in the N-terminal direction, up to 30 of amino acid residues 333 to 362 of human APP and, in the C-terminal direction, up to 30 of amino acid residues 327 to 328 of human APP, the whole forming a reverse-order sequence relative to human APP.

In formula (IV), X_1 is preferably from zero to 20

and/or X_2 is from zero to 20. More preferably, X_1 is from zero to 10 and/or X_2 is from zero to 10. Still more preferably, X_1 and/or X_2 is zero.

In other preferred compounds of formula (IV), X_1 is 2 or less and X_2 is 8 or less.

When X_1 and X_2 are both zero formula (IV) represents the polypeptide

Ser-Met-Arg-Glu-Arg (SEQ ID No: 4)

which may also be represented as SMRER and is the reverse-order sequence of the polypeptide RERMS (SEQ ID No. 3).

The invention also provides a pharmaceutical composition containing as an active ingredient a compound according to formula (IV), together with a pharmaceutically acceptable carrier, filler or excipient.

Further, the invention provides a compound according to formula (IV) which is chemically or physically linked or conjugated to a further molecule or vehicle whereby the compound can be delivered across the blood-brain barrier.

The invention also provides pharmaceutical compositions containing such compounds together with a pharmaceutically acceptable carrier, filler or excipient.

In each case, the preferred compound according to formula (IV) is

Ser-Met-Arg-Glu-Arg (SEQ ID No: 4)

The invention moreover provides a pharmaceutical composition containing as an active ingredient the polypeptide

Arg-Glu-Arg (SEQ ID No: 9)

together with a pharmaceutically acceptable carrier, filler or excipient. This polypeptide may also be represented as RER. The polypeptide RER is preferably chemically or

physically linked or conjugated to a further molecule or vehicle whereby the compound can be delivered across the blood-brain barrier. The invention also provides a pharmaceutical composition containing such a compound together with a pharmaceutically acceptable carrier, filler or excipient.

Also provided by the invention are methods of treating or preventing a neurodegenerative disease, for example Alzheimer's disease, in an animal, preferably a human, the method comprising:

identifying an animal suffering or potentially suffering from a neurodegenerative disease; and

administering to the animal an amount of at least one of the following compounds effective to treat or at least partially prevent the neurodegenerative disease,

the said compounds being:

- a compound according to formula (IV), including all preferred forms thereof, particularly Ser-Met-Arg-Glu-Arg (SEQ ID No. 4);
- Arg-Glu-Arg (SEQ ID No: 9);
- any of the foregoing compounds chemically or physically linked or conjugated to a further molecule or vehicle whereby the compound can be delivered across the blood-brain barrier; and
- pharmaceutical compositions containing as an active ingredient any of the foregoing compounds, including such compounds linked or conjugated as stated, together with a pharmaceutically acceptable carrier, filler or excipient.

Further, the invention provides a method of producing a cognitive enhancement effect in an animal, preferably a human, the method comprising:

providing an animal in which such an effect is to be produced; and

administering to the animal an amount of at least one of the following compounds effective to produce the cognitive enhancement,

the said compounds being:

- a compound according to formula (IV), including all preferred forms thereof, particularly Ser-Met-Arg-Glu-Arg (SEQ ID No: 4);
- Arg-Glu-Arg (SEQ ID No: 9);
- any of the foregoing compounds chemically or physically linked or conjugated to a further molecule or vehicle whereby the compound can be delivered across the blood-brain barrier; and
- pharmaceutical compositions containing as an active ingredient any of the foregoing compounds, including such compounds linked or conjugated as stated, together with a pharmaceutically acceptable carrier, filler or excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings of this specification consist of the following:

Figure 1 which shows the amino acid sequence of human APP and chick APP, as referred to hereinbefore;

Figure 2 shows the effect of β-amyloid 12-28 polypeptide on memory formation;

Figure 3 shows the effect of RERMS on β-amyloid 12-28 induced amnesia;

Figure 4 shows the effect of RERMS on anti-APP induced amnesia;

Figure 5 shows the effect of RERMS, SMRER and RSAER on APP-antisense induced amnesia;

Figure 6 shows the effect of APP 319-335 on APP-antisense induced amnesia;

Figure 7 shows the effect of RERMS on weak training;

Figure 8 shows the effect of APP 319-335 on weak training; and

Figure 9 shows the effect of RER on weak training.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described further by way of example with reference to the following experimental procedures and results.

Materials and methods

Animals and training

Commercially obtained Ross Chunky eggs were incubated and hatched in brooders and held until 16 ±6 hours old. Chicks were placed in pairs in small aluminium pens. Following an equilibration period of an hour, the chicks were pretrained and trained essentially as described by Lossner and Rose (J. Neurochem. 41, 1357-1363 (1983), the contents of which are incorporated herein by reference). Pretraining involved three 10 s presentations of a small (2 mm diameter) white bead, at approximately 5 minute intervals. Chicks, which failed to peck the bead at least twice in three presentations (less than 5%), were not used subsequently, but remained in their pens for the duration of the experiment. Two training techniques were used: "strong" and "weak" training. In both, 5 to 10 minutes after the last pre-training trial, chicks were trained by a 10 s presentation of a 4 mm diameter chrome bead, which had been dipped in the bitter-tasting methylanthranilate. Control chicks pecked at a water-coated or dry bead. In the "strong" version of the task, 100% methylanthranilate was used. In the "weak" version, 10% methylanthranilate was used. Chicks spontaneously pecked at the training or control beads within 20 s. Chicks that peck at the bitter bead evinced a disgust reaction and would not normally peck at a similar, but dry bead for some hours subsequently. At various times following training chicks were tested, by offering them a dry 4 mm diameter chrome bead, followed 10 minutes later by a small (2 mm diameter) white bead, each for 20 to 30 s. Animals were tested by an experimenter blind as to which treatment each chick had received. Chicks are considered to remember the task if they avoid the chrome bead at test but peck at the white bead

(discriminate), and to have forgotten it if they peck at both beads. Recall is calculated as a percent avoidance score(percentage of chicks which avoid the chrome bead) and as a discrimination score (percentage of chicks which avoid the chrome but peck at the white bead). The use of the discrimination score ensures that chicks can indeed see and peck accurately at the bead; and hence that the avoidance of the chrome bead is not due to non-specific factors such as lack of visuo-motor coordination, motivation, attention, arousal, etc. but is a positive act, demonstrating memory for the distasteful stimulus. Each chick was trained and tested only once and differences between groups tested for statistical significance by g-test described by Sokal and Rohlf (Biometry: the Principles and Practice of Statistics in Biological Research (2nd edition), W H Freeman, New York (1981)), the contents of which are incorporated herein by reference. The validity of this particular training task used to assess memory formation is extensively discussed by Andrew (Neural and Behavioural Plasticity: the Use of the Domestic Chick as a Model, Oxford University Press, Oxford, UK (1991), the contents of which are incorporated herein by reference.

Chicks trained on the strong version of the task were found to recall the avoidance for at least 48 hours, and more than 80% were found normally to avoid and discriminate on test at 24 hours. Therefore if agents that are amnesic - that is, cause the chick not to remember - are administered, chicks will demonstrate forgetting by pecking rather than avoiding the chrome bead on test. By contrast, chicks were found normally to remember the "weak" version of the task for only a few hours - some 6 to 8 hours in all; retention at 24 hours was normally reduced to some 20 to 30%. Thus the learning experience is not committed to long-term memory. Agents that are memory enhancers can thus be tested. A memory enhancing agent, administered to a chick trained on the weak learning task, produces an increase in retention - increased avoidance of the chrome

bead - at 24 hours. That is, such memory enhancers help convert weak to strong learning by enabling the transition from shorter to longer-term memory.

Peptide injections

Bilateral intracranial injections (2 μg/hemisphere) of either saline, or solutions in saline of different peptides (0.5 to 5 μg/hemisphere) homologous to different regions of the external domain of human APP were injected intracerebrally into a specific brain region, known to be required for memory formation (the intermediate hyperstriatum ventrale) at different time-points pre- or post-training using a 5μg Hamilton syringe fitted with a plastic sleeve to allow a penetration of 3 mm. After completion of the injection, the needle was kept in place for 5 s. Correct placement was ensured by using a specially designed headholder described by Davis et al (Physiol. Behav. 22, 177-184 (1979), the contents of which are incorporated herein by reference) and was routinely visually monitored postmortem. Peptides or other substances were administered at various times either before or after the training protocol. Chicks were tested at different time points post-training as described above. The general behaviour of the chicks following injections was observed to detect any potential non-specific or adverse reactions to the injections.

Peptide Materials

The polypeptides administered were synthesised using a conventional peptide synthesiser in a manner which is well-known to those skilled in the art. The synthesised polypeptides were purified by use of RP-HPLC and purity further checked by mass spectrometry (MALDI-TOF), both techniques being well known to those skilled in the art. The polypeptides after synthesis were kept under argon in a lyophilised state, the argon preventing oxidation of cysteine, methionine and tryptophan in particular.

Polypeptide synthesis as just mentioned is carried out by MWG-Biotech UK Limited of Milton Keynes, UK.

RERMS is also available from Bachem Limited of St. Helens, Merseyside, UK.

Experimental results

It is well known in many animal model systems for the study of memory that injection of β-amyloid and β-amyloid peptides, such as β-amyloid 12-28, results in a failure of animals to retain recently acquired memories. Figure 2 shows this result for a chick; injection of β-amyloid 12-28 into the brain 30 minutes prior to training chicks on the passive avoidance task results in amnesia in animals tested 30 minutes subsequently.

Figure 2 shows in the left-hand half the percent avoidance measured in terms of total avoidance and discrimination for a saline control and in the right-hand half the percent avoidance measured when β-amyloid 12-28 is injected as described above 30 minutes pretraining and memory is tested 30 minutes posttraining.

However, if amnesia is induced by injection of β-amyloid 12-28 30 minutes pretraining, and RERMS is injected 20 minutes pretraining, memory retention is restored. Figure 3 shows that in this case memory is normal at 24 hours post-training.

Figure 3 shows on the left the percent avoidance measured in terms of total avoidance and discrimination for a saline control, in the centre the corresponding results when β-amyloid 12-28 is injected 30 minutes pretraining and memory tested 24 hours posttraining, and on the right the results when the pretraining injection of β-amyloid 12-28 is followed 10 minutes later by RERMS and memory is again tested 24 hours posttraining.

It is thus shown that RERMS can prevent the memory loss produced by β-amyloid 12-28, a component of the amyloid plaques characteristic of Alzheimer's disease.

It is known that disrupting the normal function of APP

by blocking its external domain with a specific monoclonal antibody (mb22C11) around the time of training, whilst without effect on the ability of chicks to learn the passive avoidance response, prevents the transition to long term memory. The monoclonal antibody mb22C11, available from Boehringer-Mannheim, specifically recognises an epitope consisting of part of the external domain of APP.

Figure 4 shows on the left the percent avoidance measured for chicks injected with a saline control, in the centre the percent avoidance measured when mb22C11 is injected (1-5 μg in 2 μl) intracerebrally as described hereinbefore for peptide injections 30 minutes pretraining and, on the right, the percent avoidance measured when RERMS is also injected 25 minutes after mb22C11 (5 minutes pretraining). In all cases, memory was tested 24 hours posttraining.

The results shown in figure 4 demonstrate that RERMS injected 5 minutes before training will prevent antibody induced memory loss and that the peptide RERMS can prevent anti-APP induced memory loss. Thus, RERMS can prevent the memory loss resulting from disrupting the normal function of APP.

Figures 5 and 6 show the effect of inducing memory loss by injection of a 16-mer end-protected phosphodiester oligodeoxynucleotide designed to correspond to the transcription start sites 146 and AUG_{1786} of the APP mRNA, immediately upstream of a ribozyme binding site. The oligodeoxynucleotide, 5'-CCC GAG GAC TGA GCC A-3' (SEQ ID No:9) was further modified on the 2nd and 13th nucleotides to prevent internal looping and is available from King's College Molecular Medicine Unit, London, UK. The oligodeoxynucleotide was used in scrambled (SC) and antisense (AS) forms and administered as described hereinbefore for peptide administration in an amount of 0.6 to 1.0 μg in 2 μl.

In a first experiment, chicks were injected with saline, RERMS, SMRER and RSAER in various combinations in

the amounts stated hereinbefore.

The results are shown in figure 5 which shows the percent avoidances measured on the "strong" learning task described hereinbefore. Figure 5a shows the effect compared with a saline control of administration separately of SC oligodeoxynucleotide 12 hours pretraining and AS oligodeoxynucleotide 12 hours pretraining, together with the effect of administration of RERMS following the AS oligodeoxynucleotide 30 minutes pretraining.

Figure 5a shows that the SC oligodeoxynucleotide had no effect on memory but the AS compound had a significant effect of memory loss which was avoided to a substantial extent when RERMS was administered.

Figure 5b shows that similar results were obtained with the reverse-order pentapeptide SMRER.

Figure 5c shows that the effect obtained with RERMS and SMRER is absent with the pentapeptide RSAER.

In a second experiment, SC and AS oligodeoxynucleotides were administered 12 hours pretraining. A polypeptide (APP 319-335) corresponding to amino acid residues 319 to 335 of human APP was injected 30 minutes pretraining. Chicks were tested for memory according to the "strong" version of the test described hereinbefore 30 minutes posttraining.

Figure 6 shows successively from the left: the percent avoidance measured for a saline control; the percent avoidance measured when SC oligodeoxynucleotide was administered; the percent avoidance measured when AS oligodeoxynucleotide was administered; and the percent avoidance measured when APP 319-335 was administered 30 minutes pretraining following administration of AS oligodeoxynucleotide 12 hours pretraining. Each result is shown both in terms of total avoidance (left-hand column) and discrimination (right-hand column).

The results shown in figure 6 demonstrate that APP319-335 can prevent antisense induced memory loss.

Figures 7 and 8 show the effects of RERMS and APP 319-

335 on memory in chicks trained on the "weak" memory test described hereinbefore.

As stated hereinbefore, weakly trained chicks (trained on 10% methylanthranilate) retain memory for the avoidance for only some 6 hours, and thereafter forget. Figure 7 shows, on the left, the percent avoidance results (in terms of total avoidance and discrimination) for chicks trained on the "strong" version of the training, in the centre the corresponding results for "weak" training and, on the right, the effect of administration of RERMS following "weak" training. The chicks were tested for memory 24 hours posttraining; RERMS was administered in accordance with the procedure described hereinbefore 30 minutes pretraining.

Figure 8 shows the corresponding results obtained when the APP 319-335 polypeptide was used instead of RERMS.

Figures 7 and 8 show that RERMS and APP 319-335 if injected prior to training chicks on the weak task, enhance memory at 24 hours. They thus function as cognitive enhancers (nootropic agents). Thus, RERMS and APP 319-335 both enhance normal memory in weakly trained animals.

Figure 9 shows the effect of RER on memory in chicks trained on the "weak" memory test described hereinbefore.

As stated hereinbefore, weakly trained chicks (trained on 10% methylanthranilate) retain memory for the avoidance for only some 6 hours, and thereafter forget.

Figure 9 shows, in the three columns on the left, on the left the percent avoidance results (in terms of total avoidance) for chicks trained on the "strong" version of the training, in the centre the corresponding results for "weak" training and, on the right, the effect of administration of RER following "weak" training. The chicks were tested for memory 24 hours posttraining; RER was administered in accordance with the procedure described hereinbefore 30 minutes pretraining.

Figure 9 shows, on the right, the corresponding data in terms of discrimination.

Figure 9 shows that RER, if injected prior to training chicks on the weak task, enhances memory at 24 hours. It thus functions as a cognitive enhancer (nootropic agent). Thus, RER enhances normal memory in weakly trained animals.

The role of APP in memory formation has been attributed to its involvement in cell-to-substrate adhesion processes. The data reported suggests that the APP involvement in memory formation most probably involves change in signal transduction events. The post-training time within which the antibody and antisense-induced amnesia, and within which RERMS and SMRER prevents amnesia, corresponds to that during which memory formation is vulnerable to disruption of the putative signal-transduction functions of APP.

The chick system is a good one for exploring these issues, because the learning task is precise and sharply timed, and permits one also to be sure that any observed effect of an injected substance is specific to retention and not either to acquisition or to concomitant processes such as visual acuity, arousal or motor activity. Further, the role of other cell adhesion molecules in the cascade leading to synaptic modulation has been well mapped, so that the effects of either blocking or attempting to rescue functional APP activity can be set into an established context : see Rose, Learn. Memory 7, 1-17 (2000) the contents of which are fully incorporated herein by reference.

It is therefore indicated by the experimental results reported above that compounds of the present invention are effective for the treatment and/or prevention of neurological diseases and disorders and as cognitive enhancers (nootropic agents) in other animals, including human and non-human mammals. The compounds are therefore effective in the treatment and/or prevention of Alzheimer's disease in humans and other neurodegenerative diseases and disorders in animals generally, including humans. Such animals include transgenic and other animal models for

Alzheimer's disease.

As used herein, except where the context indicates otherwise, the terms "treatment", "treat" and analogous expressions used in relation to neurodegenerative diseases include within their scope not only treatment when symptoms are apparent but also the partial or total prevention of such diseases and delay in their onset in patients or animals who are subjected to treatment before onset of the disease or its symptoms become apparent.

The compounds may be administered intracerebally as described above, or may be administered peripherally, for example intramuscularly, intravenously, transdermally or orally, preferably after complexation as described above. Instead or in addition, the compounds may be protected against alteration between administration and effectiveness, for example by addition of protective groups.

The compounds may also be formulated as pharmaceutical compositions as referred to hereinbefore, particularly such compositions as are capable of crossing the blood-brain barrier and thereby suitable for peripheral administration.

In all events a suitable dose of peptide compounds according to the invention is from 10 to 100 μg/kg body weight of the animal being treated.

As used herein, the term "effective to treat" in the context of a neurodegenerative disease means that amount of the compound(s) used in the treatment which causes a reduction or stabilisation or, as the case may be, prevents or delays the appearance of such symptoms as measured by standard medical or psychological criteria, for example as disclosed in Handbook of

Memory Disorders (eds: A D Baddeley, B A Wilson and F N Watts), Wiley (1995), the disclosure of which is herein incorporated by reference.

As used herein, the term "effective to treat" in relation to a cognitive enhancement means an amount of the compound(s) used in the treatment which causes an improvement in cognitive power as measured by psychological criteria, for example as disclosed in Handbook of Memory Disorders (eds: A D Baddeley, B A Wilson and F N Watts), Wiley (1995), the disclosure of which is herein incorporated by reference.